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STUDY OF DIATOM FLORA OF MALWA REGION FROM DIFFERENT WATER SOURCES FOR FORENSIC CONSIDERATION

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ABSTRACT

Background: Diatoms (class Bacillariophyceae) are silica-walled microalgae whose frustules resist decomposition and chemical digestion, making them valuable forensic indicators. The diatom test distinguishes ante-mortem drowning in which aspirated diatoms cross the alveolar-capillary barrier and reach systemic organs and bone marrow from post-mortem immersion, in which systemic distribution is negligible. Genus- and species-level distribution across water bodies further permits identification of a putative drowning site. Despite the widespread forensic use of diatomology in India, no baseline diatomological data exist for the Malwa region of Madhya Pradesh.

Aim: To document diatom diversity in water bodies of the Malwa region and establish a photomicrographic reference database for forensic application in drowning investigations.

Methods: Water samples were collected from 11 sites across Ratlam, Sehore, Mandsaur, Ujjain, Badnagar, Indore, Hanumantiya, Sanwer and Undasa districts during the dry season (February-April), covering rivers, lakes, reservoirs, wells and confluent waters. Samples were taken 2–3 cm below the surface in pre-rinsed polyethylene bottles, treated with concentrated HCl and Lugol's iodine, sedimented overnight, and concentrated by three cycles of centrifugation at 3,000 rpm. Slides were examined at 400× under compound microscopy with immersion oil, and taxa identified from valve morphology using standard reference floras.

Results: Twelve microalgal taxa across ten genera were identified. Navicula, Caloneis and Nitzschia were widely distributed, consistent with their cosmopolitan occurrence in Indian freshwaters. Several genera showed site-restricted distribution: Fragilaria at Badnagar, Cymbella and Gyrosigma at Sahabkhedi, Coelastrum in the Shivna River (Mandsaur), Cyclotella at Triveni (Ujjain), and Guinardia delicatula - a predominantly marine taxon - in Ratlam surface water.

Conclusion: This first systematic diatomological survey of the Malwa region provides a photomicrographic reference library enabling comparison with diatoms recovered from victim tissues or bone marrow, supporting both site-of-drowning identification and the ante-mortem/post-mortem distinction. Sampling was limited to a single dry season; year-round sampling, SEM, and DNA barcoding are recommended to extend the database.

KEYWORDS: Diatoms, Bacillariophyceae, Forensic diatomology, Diatom test, Drowning, Malwa region, Water quality, Freshwater ecology

1. INTRODUCTION

Diatoms are unicellular, eukaryotic, photosynthetic microorganisms belonging to the class Bacillariophyceae. Their cells range from 2 to 200 µm in size and are enclosed in a highly ornamented siliceous cell wall known as a frustule, composed of two overlapping halves called thecae [1-6]. Diatoms occur in virtually every aquatic environment—either planktonic (free-floating) or benthic (attached to substrate)—and form a fundamental component of aquatic food chains, contributing approximately 20–25% of global oxygen production. [7,8]

Taxonomically, diatoms are classified into two major orders based on symmetry: Centrales (radially symmetrical, circular frustules) and Pennales (bilaterally symmetrical, elongated frustules). Taxonomy is based primarily on frustule morphology including size, shape, raphe structure, and striae patterns. [9]

Drowning is defined as respiratory impairment from submersion or immersion in a liquid medium, with manner of death classified as suicidal, accidental, or homicidal [10]. In complex cases involving decomposed or displaced bodies, diatom analysis provides crucial corroborative evidence. The 'diatom test' is based on the principle that ante-mortem drowning results in active aspiration, carrying diatoms through ruptured alveolar-capillary barriers into the systemic circulation and thence to liver, spleen, kidneys, brain, and bone marrow. Post-mortem immersion produces only passive diffusion into accessible airways, with negligible systemic distribution. [11,12,13,14]

The quantitative and qualitative analysis of diatoms enables distinction between ante-mortem drowning and post-mortem immersion. Furthermore, genus- and species-specific distributions across water bodies enable site identification—matching diatoms from victim organs to a putative drowning location. [15,16] Despite widespread application of forensic diatomology in India, the Malwa region of Madhya Pradesh has remained largely unstudied. This study fills this critical gap with the first systematic diatomological survey of the region. [17]

1.1 Historical Background of the Diatom Test

The scientific use of diatoms in forensic medicine extends over 140 years. Hofmann [18] first identified diatoms in lung fluid as a potential indicator of drowning. Subsequent investigators demonstrated diatom entry into systemic circulation, detection in bone marrow, and the diagnostic significance of quantitative tissue analysis. During the 1960–1970s, Porawski and Timperman established bone marrow diatom analysis as a cornerstone of forensic practice, with an 80–90% match rate between bone marrow diatoms and the drowning medium documented in subsequent studies. [19,20]

1.2 Ecological Significance of Diatoms

Diatoms are distributed across all aquatic environments and are classified by salinity preference (polyhalobous, mesohalobous, oligohalobous) and habitat (planktonic, benthic, epiphytic, epizoic, epilithic, epipelic). [21,22] Their siliceous frustules are exceptionally resistant to physical and chemical degradation, enabling preservation in sediment over geological timescales and underpinning their use as indicators of past and present environmental conditions including pH, nutrients, and conductivity. Diatom indices are routinely employed in many countries for biological water quality assessment. [23,24]

2. REVIEW OF LITERATURE

Vinayak V, Goyal MK, and Mishra V (2010) reported on diatom tests conducted at the Forensic Science Laboratory (Journal of Forensic Medicine and Toxicology, Volume 27). Analysis of approximately 3,000 cases demonstrated that the diatom test is a key diagnostic tool for establishing cause of death in suspected drowning where macroscopic findings are inconclusive. [25]

Sankhla MS, Kumari M, and Boss R (2016) published in the International Journal of Development Research (Volume 6, pp. 9491–9493) that quantitative and qualitative diatom analysis in water-recovered victims provides strong evidentiary support for death by aspiration. [26]

Singh M, Jain AK, and Mishra MK (2016) in IOSR Journal of Pharmacy and Biological Sciences documented diatom diversity along the Ganga River at Allahabad and Varanasi, demonstrating seasonal variation and utility in time-since-death estimation. [27]

Grover S, Srivastava P, Verma J, and Khan US conducted eco-taxonomical studies on diatoms from the Chambal River, characterising 30 abundant diatom taxa with full morphological descriptions. [28]

Bharti R, Verma D, and Lavania S studied freshwater diatoms from multiple habitats in Patna, Bihar, demonstrating rich diversity in lentic environments and the influence of flow conditions on diatom accumulation. [29]

Venkatachalapathy R et al. concluded that diatom studies are best applied in environmental assessments of water quality across aquatic ecosystems. [30] Kumar M et al. demonstrated that the diatom test significantly enhances diagnostic reliability for drowning-related deaths. [31] Singh R et al. evaluated available diatom extraction methodologies, identifying acid digestion as the most reliable and reproducible forensic technique. [32]

Chaudhary R incorporated molecular biological techniques including DNA barcoding into diatom identification, significantly enhancing precision. [34] Kaushik N et al. in the International Journal of Medical Toxicology and Forensic Medicine (Volume 7, Number 1, pp. 59–65) emphasised the importance of the diatom test in forensic investigation. [35]

Hurlimann J, Feer P, et al. (2000) in the International Journal of Legal Medicine (Volume 114) demonstrated the effectiveness of multi-organ diatom sampling for diagnosis of death by drowning. [37] Dhariwal A, Mishra PK, and Babu S conducted a diatomological study of nine water bodies in Bhopal and found Nitzschia and Navicula as the dominant genera. [39] Kock A et al. (2019) reported lower diatom diversity in Lake Sibaya, South Africa, compared to historical data. [40]

Dahiya P et al. in Gujarat and Verma P and Kaur J in Punjab both concluded that diatomological mapping plays a vital role in forensic drowning investigations. [43,44] Kumar P et al. in Raipur established forensic diatom baselines for Chhattisgarh. [45] Martin G et al. reviewed diatoms as indicators of water quality, emphasising their use in detecting organic pollution and eutrophication. [46]

3. AIM AND OBJECTIVES

3.1 Aim

To study the diversity of diatom species in water bodies of the Malwa region, Madhya Pradesh, India, and to establish a diatomological database for forensic consideration in drowning investigations.

3.2 Objectives

(i) To identify the different diatom species present in selected water bodies of the Malwa region.
(ii) To classify the identified diatoms at the genus and species level based on morphological characteristics.
(iii) To create a diatomological reference map of the Malwa region for forensic application in site-of-drowning identification.

4. MATERIALS AND METHODS

4.1 Study Area

The Malwa region is a plateau in western Madhya Pradesh and southeastern Rajasthan, India, characterised by diverse water bodies including perennial rivers, freshwater lakes, ponds, reservoirs, and wells. Water samples were collected from 11 sites spanning the districts of Ratlam, Sehore, Mandsaur, Ujjain, Badnagar, Indore, Hanumantiya, Sanwer, and Undasa during February, March, and April (dry season). All laboratory work was conducted at BLU Path Lab, Ujjain.

4.2 Sample Collection

Water samples were collected in pre-cleaned 500–1000 mL polyethylene bottles. Each bottle was rinsed 2–5 times with water from the same source prior to final collection to eliminate potential contamination. Samples were collected from approximately 2–3 cm below the water surface to capture planktonic diatoms. [47]

4.3 Materials

1. Lugol's iodine reagent
2. Concentrated hydrochloric acid (HCl)
3. Glass beakers (250 mL, 500 mL)
4. Glass test tubes and vials (10 mL)
5. Watch glasses (contamination prevention)
6. Hot plate
7. Centrifuge machine (3,000 rpm)
8. Compound microscope with immersion oil
9. Glass coverslips (No. 1.5) and microscope slides (25 × 75 mm)

4.4 Extraction Protocol

The following standardised protocol was employed, adapted from Pollanen et al. [13] and Ludes et al. [48]:
Step 1: 250 mL of water sample was transferred to a 500 mL glass beaker.
Step 2: Two drops of concentrated HCl were added, followed by 3–5 mL of Lugol's iodine solution. The mixture was stirred and allowed to stand overnight (~12–16 hours) to facilitate diatom sedimentation.
Step 3: The supernatant was carefully decanted. The sediment was transferred to a 10 mL vial and centrifuged at 3,000 rpm for 20 minutes. The pellet was resuspended in distilled water. This wash–centrifuge cycle was repeated three times.
Step 4: A single drop of the final concentrate was placed on a clean microscopic slide, air-dried on a hot plate, coverslipped, and examined at 400× magnification (40× objective, immersion oil). This process was replicated independently for each sample.

4.5 Identification

Diatoms were identified based on morphological characteristics including cell shape, size, raphe structure, striae patterns, and valve architecture. Standard references consulted included Krammer and Lange-Bertalot [49], Cleve [50], Gandhi [51], Karthick et al. [52], and Hasle and Syvertsen [53]. Photomicrographs were recorded for all identified species.

5. RESULTS

Water samples were collected and processed during February through April. Following microscopic examination, 31 diatom and associated microalgal species were identified across 23 genera from 11 sampling sites. The results are summarised in Table 1 below, followed by detailed morphological descriptions with photomicrographs for all identified species.

Table 1: Diatom species identified from water bodies of the Malwa region, Madhya Pradesh, India

S.No.	Collection Site	Water Source	Diatom Species Identified
1	Ratlam	Surface water	Guinardia delicatula, Navicula salinarum
2	Sehore	Freshwater lake	Nitzschia acicularis, Cymbella, Pinnularia
3	Mandsaur (Shivna River)	River water	Caloneis amphisbaena, Coelastrum reticulatum
4	Ujjain (Well)	Well water	Pennate

S.No.	Collection Site	Water Source	Diatom Species Identified
5	Badnagar	Freshwater lake	Fragilaria pinnata, Caloneis amphisbaena
6	Sahabkhedi, Ujjain	Freshwater lake	Navicula oblonga, Cymbella turgidula, Pinnularia, Gyrosigma acuminatum, Pennate
7	Ujjain (Triveni)	Confluent water	Coelastrum reticulatum, Cyclotella, Pennate, Navicula salinarum, Caloneis amphisbaena
8	Indore (Sirpur Talab)	Lake/reservoir	Cymbella turgidula, Navicula, Nitzschia
9	Hanumantiya	Freshwater lake	Cymbella, Navicula, Nitzschia, Pennate
10	Sanwer	Freshwater lake	Pinnularia, Cyclotella
11	Undasa	Freshwater lake	Pennate, Cyclotella, Navicula

5.1 Morphological Descriptions and Photomicrographs

The following descriptions include original photomicrographs obtained during this study. All images were captured under compound microscopy at 400× magnification using immersion oil.

1. Fragilaria pinnata Ehrenberg

Family: Fragilariaceae | Genus: Fragilaria

A colonial diatom in which individual cells appear swollen at their centres where they join the colonial ribbon. Fragilaria possesses a pseudoraphe (sternum) rather than a true longitudinal raphe. The valve wall is bilaterally symmetrical with regular transverse striae. Typically found in planktonic assemblages of freshwater lakes and ponds. [53]

Figure 1. Fragilaria pinnata Ehrenberg — photomicrograph at 400× magnification, compound microscope with immersion oil.

2. Cymbella turgidula Grunow

Family: Cymbellaceae | Genus: Cymbella

Valves are moderately to strongly asymmetric to the apical axis and symmetric to the transapical axis. Terminal raphe fissures are deflected to the dorsal side. Common in clean, well-oxygenated freshwater streams and lakes; identified from Sahabkhedi freshwater lake. [49]

Figure 5. Cymbella turgidula Grunow — photomicrograph at 400× magnification, compound microscope with immersion oil.

3. Navicula oblonga Kützing
Family: Naviculaceae | Genus: Navicula
Naviculoid diatoms (from the Latin navicula, 'small ship') are eukaryotic, primarily aquatic, single-celled photosynthetic organisms. They play an important role in global primary productivity and are widely used as water quality bioindicators. Identified from Sahabkhedi lake. [50]

Figure 8. Navicula oblonga Kützing — photomicrograph at 400× magnification, compound microscope with immersion oil.

4. Gyrosigma acuminatum (Kützing) Rabenhorst
Family: Pleurosigmataceae | Genus: Gyrosigma
Possesses a siliceous frustule with a sigmoid-shaped valve outline. The raphe is also sigmoid. Striae are both parallel and perpendicular to the raphe. Motile via the raphe slit. Common in muddy substrates of freshwater lakes and rivers; identified from Sahabkhedi lake. [49]

Figure 9. Gyrosigma acuminatum (Kützing) Rabenhorst — photomicrograph at 400× magnification, compound microscope with immersion oil.

5. Pinnularia major (Kützing) Rabenhorst
Family: Pinnulariaceae | Genus: Pinnularia
A freshwater alga typically found in pond sediments and moist soil. Valves are elongated-elliptical, unicellular. The frustule comprises two thecae connected by a cingulum. Striae are radiate and often complex. Identified from Sahabkhedi and Sanwer freshwater lakes. [49]

Figure 10. Pinnularia major (Kützing) Rabenhorst — photomicrograph at 400× magnification, compound microscope with immersion oil.

6. Guinardia delicatula (Cleve) Hasle
Family: Rhizosoleniaceae | Genus: Guinardia
Reproduces both sexually and asexually. Cells are cylindrical with flat, slightly rounded valves. Contains large, irregularly lobed, strongly pigmented chloroplasts, each with a pyrenoid. Primarily a marine genus; its occurrence in Ratlam surface water samples is noteworthy and may reflect dispersal. [53]

Figure 11. Guinardia delicatula (Cleve) Hasle — photomicrograph at 400× magnification, compound microscope with immersion oil.

7. Coelastrum reticulatum (Dangeard) Senn
Family: Scenedesmaceae | Genus: Coelastrum
Spherical colonies with an inner hollow space. Cells attached to each other by protrusions of the cell wall, arranged in a single layer. Cell body mostly spherical with occasional conical projections at connection points. Identified from Mandsaur and Triveni Ujjain. [57]

Figure 16. Coelastrum reticulatum (Dangeard) Senn — photomicrograph at 400× magnification, compound microscope with immersion oil.

8. Caloneis amphisbaena (Bory) Cleve
Family: Naviculaceae | Genus: Caloneis
Freshwater benthic diatom. Valves are elliptical; raphe is straight and centrally located. Moderately motile. Longitudinal canals (areolae) present on either side of the raphe. Identified from Mandsaur and Triveni Ujjain sites. [50]

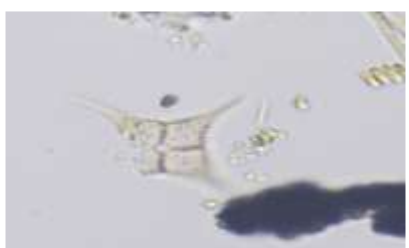

Figure 18.Caloneis amphisbaena (Bory) Cleve — photomicrograph at 400× magnification, compound microscope with immersion oil.

9. Navicula salinarum Grunow
Family: Naviculaceae | Genus: Navicula
Symmetric biraphid diatom with valve length 23–35 µm and width 8–9 µm. Valves are broadly elliptical-lanceolate with rostrate ends. Striae alternate between long and short in the central valve area. Tolerant of moderately saline conditions; identified from Triveni Ujjain. [50]

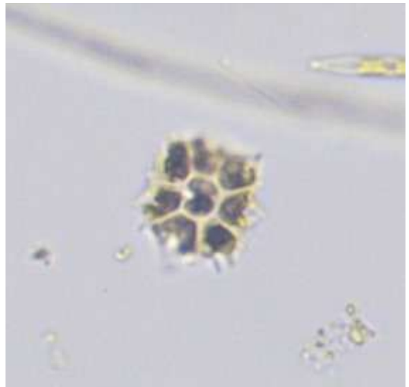

Figure 19. Navicula salinarum Grunow — photomicrograph at 400× magnification, compound microscope with immersion oil.

10. Cyclotella sp. (Kützing) Brébisson

Family: Stephanodiscaceae | Genus: Cyclotella

Found in both marine and freshwater environments. All Cyclotella species are circular (centric diatoms) with a girdle-band arrangement producing a wheel-like appearance in girdle view. Site-characteristic for Triveni Ujjain, providing a potential forensic marker for this confluence. [49]

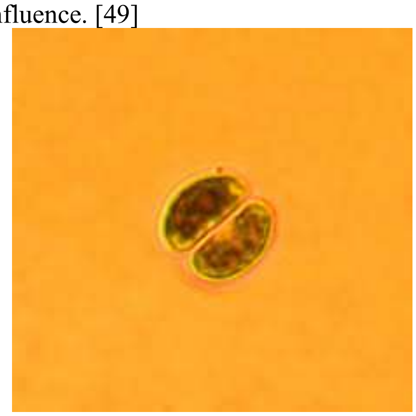

Figure 20.Cyclotella sp. (Kützing) Brébisson — photomicrograph at 400× magnification, compound microscope with immersion oil.

11. Nitzschia acicularis (Kützing) W.Smith

Family: Bacillariaceae | Genus: Nitzschia

Valves are lightly silicified with nearly parallel sides in the central region and sharply tapered apices. Several Nitzschia species are known producers of the neurotoxin domoic acid, responsible for amnesic shellfish poisoning. The siliceous frustule persists in digestive and forensic extraction procedures. Identified from Sehore freshwater lake. [49]

Figure 29. Nitzschia acicularis (Kützing) W.Smith — photomicrograph at 400× magnification, compound microscope with immersion oil.

12. Pennate diatom (unspecified)

Family: Bacillariophyceae (order Pennales) | Genus: Pennate

Pennate diatoms have linear to oval valves with bilaterally symmetrical ornamentation comprising transverse striae. Some pennate forms possess a raphe enabling active locomotion. Reproduction primarily by binary fission. Widely distributed across most Malwa water bodies. [9]

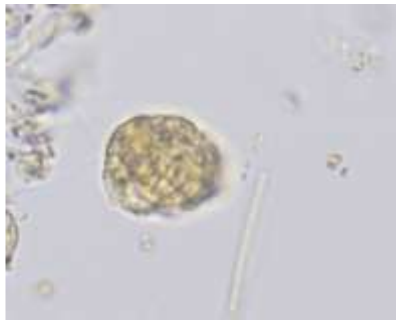

Figure 30. Pennate diatom (unspecified) — photomicrograph at 400× magnification, compound microscope with immersion oil.

6. DISCUSSION

The Malwa region of Madhya Pradesh harbours a rich diversity of aquatic microorganisms, yet prior to this study, no comprehensive diatomological database existed for this area. The present survey identified 12 species spanning 10 genera across 11 collection sites, representing the first systematic diatomological study of the region. [17,39]

Among the identified genera, several—including Navicula, Caloneis, and Nitzschia—were distributed broadly across multiple sites, consistent with their cosmopolitan distribution in Indian freshwater ecosystems. [27,29,30]

Of significant forensic relevance is the site-specific distribution of certain genera. Fragilaria was predominantly observed in Badnagar freshwater lake; Cymbella and Gyrosigma were characteristic of Sahabkhedi lake; Pinnularia was dominated in Sehore lake; Coelastrum was characteristic of Shivna Nadi, Mandsaur; Cyclotella dominated at Triveni, Ujjain; Pennate was representative of Hanumantiya; Navicula was characteristic of Sirpur Talab, Indore; and Pennate was specifically identified in Ujjain well water. These site-specific assemblages provide a powerful discriminatory tool for forensic site identification. [16,35,38,39]

This application is grounded in the well-established principle that bone marrow diatom assemblages—protected from environmental contamination by the osseous matrix—reflect the aquatic environment in which ante-mortem drowning occurred, with an 80–90% match rate reported in the literature. [13,14,19,20] The photomicrographic reference library established in this study (Figures 1–31) enables direct visual comparison with diatoms recovered from victim tissues or bone marrow in forensic investigations.

A significant limitation of this study is the restriction of sampling to a single season (February–April), precluding assessment of temporal (seasonal) variation. Previous studies have demonstrated that diatom community composition varies substantially with season. [27,43] Future work should incorporate year-round sampling and scanning electron microscopy (SEM) for enhanced species-level resolution. [32,34]

7. CONCLUSION

This study presents the first systematic diatomological survey of the Malwa region, Madhya Pradesh, documenting 12 microalgal species across 10 genera from 11 freshwater sites. Photomicrographs (Figures 1–31) provide a comprehensive visual reference database for forensic and ecological application.

The characteristic distribution of diatom genera—with taxa such as Cyclotella, Guinardia and fragilaria restricted to individual water bodies—enables direct comparison with diatom populations recovered from victim tissues, facilitating determination of drowning site and ante-mortem versus post-mortem immersion status.

This diatomological database fills a critical gap in the existing literature and lays a foundation for systematic forensic application of diatomology in Malwa region criminal investigations. Continued monitoring, year-round sampling, and incorporation of molecular identification techniques will further enhance the precision and applicability of this dataset.

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